The Microbiota Role in Health and Immunity

An Honors Thesis (HONR 499)

By

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Abstract

The study of the microbiota is a relatively new field that still has so much unknown. Until recently it was assumed by most of the population that all microorganisms were harmful. However it is becoming known now that these microorganisms have grown and evolved with humans for thousands of years. It is estimated that there are around 100 trillion microorganisms that live on and in the human body. This means that these microorganisms outnumber our human cells 10 to 1 and play a huge role in many functions of the body. These organisms also exhibit many of their own unique genes, which far outnumber the number of genes expressed by our own human cells. One of the main functions of these organisms is their role in the immune system and how it relates to health. When working at optimal level the microorganisms and body's immune system work together to fight off pathogens. In todays germ free age though the new autoimmune disorders, allergies, and other disorders are on the rise due in some part to a disruption of the body's natural microorganisms. This paper will explore the effects these microorganisms have on the immune system and other components of health. Specifically it will look at how these organisms develop and establish themselves in the body. It will also look at tolerance as well as colonization resistance and the role the microflora has in these processes. Lastly it will look at the gut/brain barrier and different disorders that can arise from a disymbiosis in the microflora.

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questions I have. I would also like to thank my parents Martha and Kim Andersen for helping and supporting me throughout the process.

Process Analysis

The process of writing this paper was very long and required a lot of research. The idea for the thesis came in my BIO 401 class where we learned about the microflora. This gave me the base of information and knowledge to begin writing the paper. I first started out reading literature reviews on different components of the microflora. As I read those I found areas that I wanted to focus on and started to look for more primary research on the subject. This research for the paper required many hours of reading journal articles, identifying important information, and synthesizing a review of topics from that information. The first papers that I read talked a lot about colonization resistance, which is a major part of the paper. Another major area of interest I discovered was how many different disorders that can result from an unhealthy microflora. For example the high use of antibiotics is increasingly linked to the development of allergies and other disorders in children as a result of an unhealthy microflora. Through the process I wanted to highlight some of the problems this can cause and stress the importance that these microorganisms have with health.

While writing this paper I also learned how to perform more efficient literature research skills and utilize the databases available at Ball State. I used a variety of different databases such as academic search premier and the One Search feature. Through the process I also learned how to use software called Endnote. This helps to generate citations and organize your sources for a research paper and will be very helpful to use on future papers. Trying to organize all the sources and information was a large challenge for the paper and something I definitely improved on while writing the paper.

The Microbiome

The microflora is a vast and very complex system. Each person has trillions of organisms living on him or her and interacting with their body. However when a person is born they are germfree and their body has to be exposed to these organisms. This means that the first exposure to these organisms comes through the birth process (Fanaro, Chierici, Guenini, & Vigi, 2003). The exposure comes as the infant passes through the birth canal and the mother is the main contributor to the colonization of these microorganisms. As the baby develops this microflora begins to take hold and expand. At first the microflora is not diverse with only a few different bacteria dominating the landscape. As the infant develops they are exposed to millions of bacteria everyday and these bacteria shape the colonization of their microflora. Most of the microflora in the gut is located in the ileum of the colon (Fanaro, Chierici, Guerrini, & Vigi, 2003). Another source of bacteria comes from the mother's breast milk during feeding (Marcobal & Sonnenburg, 2012). The breast milk is high in many oligosaccharides which *Bifidobacteria* use as an energy source. *Bifidobacteria* bacteria make up a large portion of the gut microflora and thrive in the anaerobic conditions (Marcobal & Sonnenburg, 2012). After the first week of life a more stable microflora begins to develop as the infant is exposed to more bacteria. Diet, environment, and a variety of other factors will help to shape the population of the infant's microflora. *Bifidobacteria* is one of the most abundant organisms found in the gut but many other anaerobes such as Clostridium are found as well.

The microflora takes years to establish and even after establishment still constantly changes slightly (Ringel-Kulka et al., 2013). With both children and adults the most common phylum of bacteria are the *Firmicute* bacteria making up 60-80% of the bacteria population (Ringel-Kulka et al., 2013). The second most abundant phylum between adults and children is the *Bacteriodetes*, which makes up 15-30% of the population (Ringel-Kulka et al., 2013). The third most abundant phylum was the *Actinobacteria* with the percentage of this phylum varying. The difference between children and adult populations however reflects the development of the microflora. The most abundant bacterial group found is the Clostridium, which once again was consistent for both children and adults. This could indicate that this group is established early in the development and very important to the health of the microflora early in life (Ringel-Kulka et al., 2013). In the colon there is around 50 different genera of bacteria with hundreds of different species. The establishment of this microflora still has many unknown factors. Each person has his or her own unique flora yet many people's microflora are remarkably similar. Even after a person has established a stable flora it can still change. People taking antibiotics will not only kill the pathogens but also disrupt their healthy microflora. A probiotic can also be taken but the organisms are transient and the microflora is resistant to change (de Vrese & Schrezenmeir, 2008).

The Microbiota and Immunity

The establishment of these organisms also contributes to the physical development of the immune system (Bauer, Horowitz, Levenson, & Popper, 1963). In a study done on germ free mice it showed that the mice's development of the immune system was severely inhibited. In general, germ free mice have less developed gut

associated mucosal tissue (GALT) as well as smaller secondary lymphoid structures. Peyer's Patches which is where many different antigens are sampled along the mucosal membrane using M cells were much smaller (Sato & Iwasaki, 2005). This means that fewer antigens could be sampled and these areas would be less able to create tolerance. The study also showed that these mice have less Helper T cells as well as fewer IgA producing plasma cells. The epithelial wall in these mice was also much weaker as the commensals are needed to help keep the integrity of this wall (Bauer et al., 1963). The mice have also shown to have less developed GALT as well as smaller secondary lymphoid structures. All of these structures are key to proper immune function and when not developed properly can leave the body susceptible to many diseases and disorders.

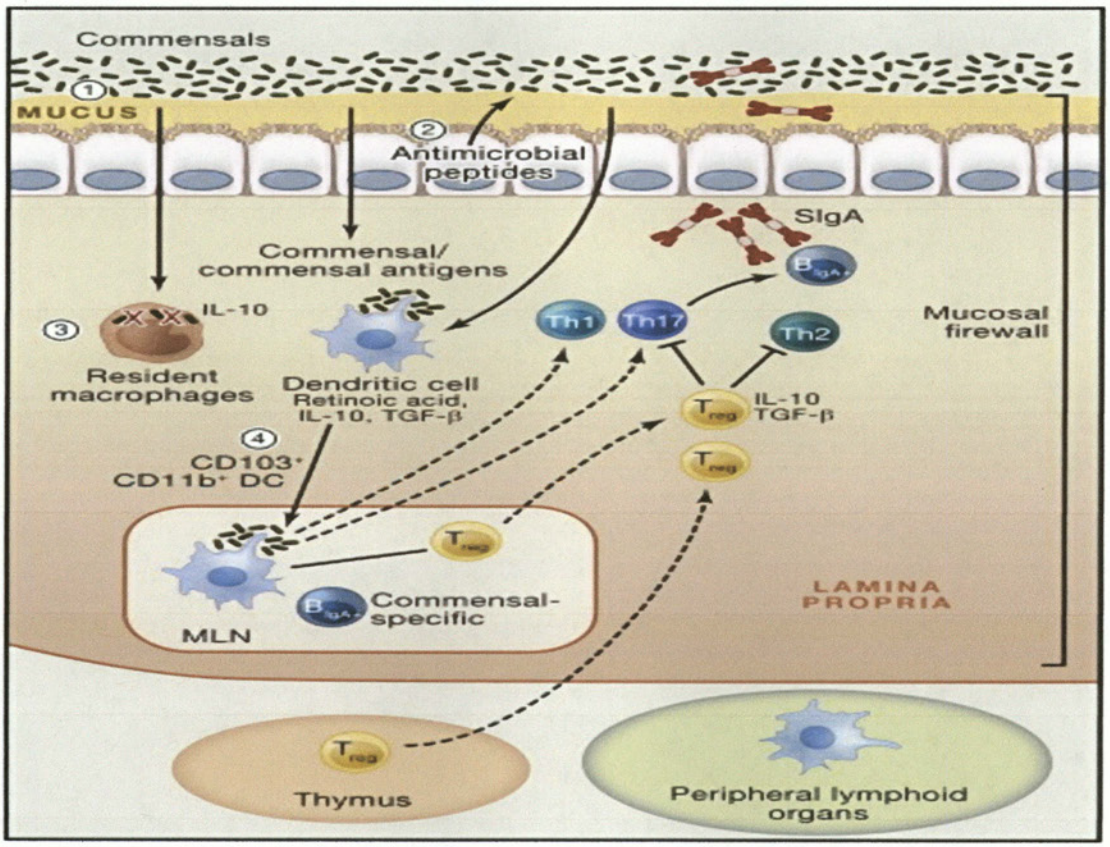

Figure 1: This image shows how mucosal immunity has specific immune cells at each site working with the microflora to contain infection while keeping an anti-inflammatory environment (Belkaid & Hand, 2014) .

The GALT and Peyers Patches are all components of what is called mucosal immunity. This mucosal immunity is largely mediated by the commensal organisms and has a unique response to pathogens. Unlike other areas of body the mucosal tissues have many anti-inflammatory properties (Brandtzaeg, 2009). The mucosal immunity plays a delicate role in trying to contain and eliminate pathogens without causing a full inflammatory response. Figure 1 shows the commensal organisms interacting with the epithelial barrier. This mucosal membrane has a large surface area and is constantly in contact with microbes. It comes in direct contact with foreign antigens as food is eaten as well as particles that are introduced in the air. This layer first has a layer of mucus that is created by goblet cells. This mucus acts as shield and prevents the microorganisms from being able to translocate across it. This mucosal membrane also has a layer of commensal organisms that also acts as a barrier. Like the microflora of the gut these also have antimicrobial properties that prevent pathogens from being able to attach (Mayer, 2003). Across this membrane the immune system is sampling different antigens and building defense against them. Many of these samples come from commensal organisms and help to create tolerance in the immune cells. These commensal are constantly sampled and their antigens are presented to create a state of tolerance in the immune cells. IL-10 is a major cytokine produced as seen in figure 1 that prevents inflammation (Ouyang, Rutz, Crellin, Valdez, & Hymowitz, 2011). Many inflammatory disorders are caused as a result of a microflora disruption that causes inflammation in the mucosal barrier.

Establishing this healthy get microflora is so important to many components of health. With infants especially microbes have a negative connotation that is associated with sickness. During this development the body continues to form the immune system

and microbes are an essential part of this development. One of the main ways they can do this is to help create tolerance. Tolerance is the body's ability to recognize certain foreign antigens as not harmful and not produce a full-scale response. As seen in figure 2 the body is in homeostasis without any response.(Adkins et al., 2011) While on the other side of the image there is inflammation that is causing damage to the surrounding tissue. During a humans life they are exposed to countless foreign antigens. Most of these are not harmful and there is no need to produce a harmful inflammation response. Inflammation causes tissue damage and can cause the epithelium barrier to be broken. Breaking this epithelial barrier allows for other opportunistic pathogens to take advantage and invade the body causing disease.

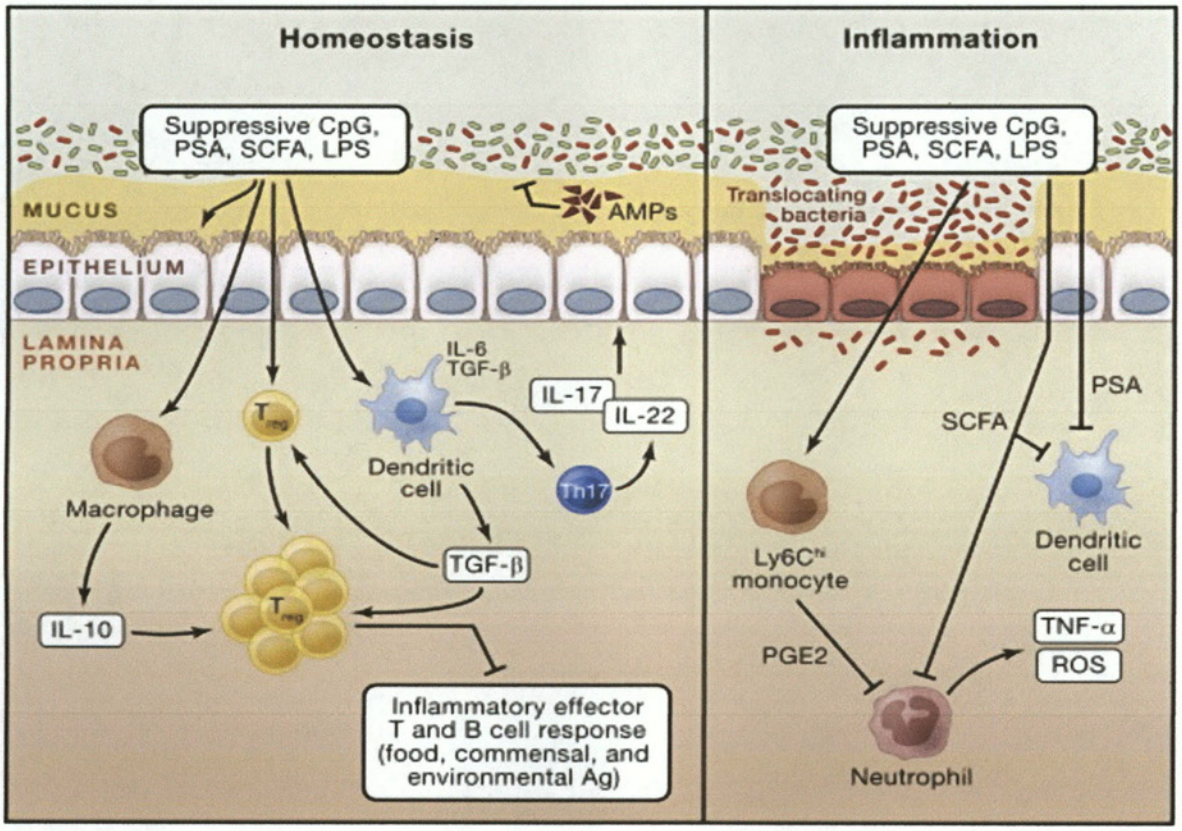

Figure 2: This image shows the difference between homeostasis and the body responding to a foreign antigen trying to limit inflammation (Belkaid & Hand, 2014).

This important process of tolerance is largely regulated by the microflora. Early exposure to the microflora helps to produce cells that are in a tolerant state. In the human body dendritic cells are constantly taking up antigens, processing them, and presenting them to various immune cells such as T cells. Interactions with the microbiota, keep dendritic cells from activating an inflammatory response when presenting harmless antigens to T cells. This regulation of cells helps to prevent many disorders that are becoming very common today.

Colonization Resistance

Colonization resistance is another major role of the microflora. This term means that these healthy microflora help to block other harmful bacteria from entering or attaching to the body. (Sassone-Corsi & Raffatellu, 2015) The microflora has many different mechanisms that it uses for colonization resistance as seen in figure 3. The first way that the microflora does this is through taking up space. On the surface of the skin there are trillions of bacteria that are attached. Their attachment prevents other harmful bacteria from being able to attach helping to protect the body (Lawley & Walker, 2013). Without this layer of commensals the pathogens would be able to attach freely to the skin or intestines and try to breach the epithelium. Additionally the lining of the intestines is a mucosal wall, which has a layer of commensal organisms that line it as well. This layer has a similar effect in that it doesn't allow pathogens to directly bind to the cells of the epithelial layer.

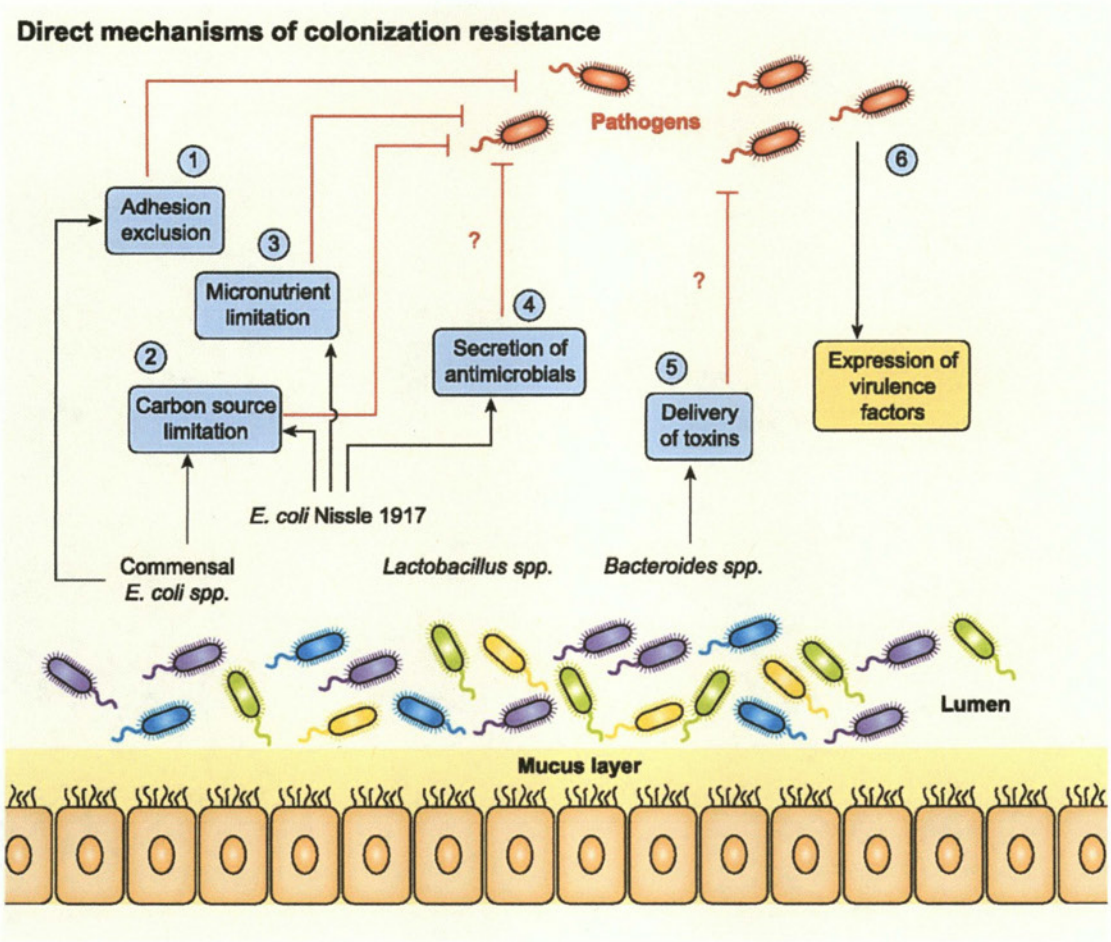

Figure 3: This figure shows the several different ways that the microflora contributes to colonization resistance (Sassone-Corsi & Raffatellu, 2015).

With many of these organisms they not only take up space but also secrete antimicrobial toxins. These toxins are meant to kill other potential harmful bacteria from colonizing helping to maintain a healthy microflora. In the bacteria *E. coli* the healthy commensal organisms produce bacteriocins that inhibit the growth of specific bacteria (Toshima et al., 2007). The commensal *E. coli* inhibits the growth of similar strains that could be pathogenic. These toxins are specific to certain bacteria and strains so they do not interfere with other normal commensal organisms. Another example of this colonization comes with *Staphylococcus epidermis* that is a common commensal organism found on the skin. This bacterium creates different kind of antimicrobial proteins that limits the

formation of *Staphylococcus aureus* from forming on the skin (Iwase et al., 2010). Without these organisms on the skin bacteria could start to form biofilms, which are much more stable and resistant then normal bacteria. The proteases created are very specific to bacteria and have evolved with bacteria and their human hosts.

Colonization continues with bacteria out competing other bacteria for certain metabolites. In the human body there can be limited competition for resources that that the bacteria use as energy (Kamada, Chen, Inohara, & Nunez, 2013). The commensal organisms that have evolved with humans have found specific nutrient's they use to carve out their niche in the human body. When foreign pathogens enter the body they enter into an environment that is hard to get nutrients. This is one way that the commensals help protect the body by out competing other pathogens for limited resources. This competition ensures that only the commensals are able to survive and the pathogens die off (Kamada et al., 2013).

In the gut these commensal bacteria can also alter the environment, which makes it harsh for pathogens to survive. In the gut there is a large population of Lactobacilli that make up the microbial flora. In their metabolic processes they create lactic acid, which is a byproduct of their cell processes. As this lactic acid is released it creates a low ph that is much to acidic for foreign pathogens to survive in (Lawley & Walker, 2013). This same environment is produced in the vaginal tract, which also has an acidic environment because of the production of lactic acid (Turovskiy, Sutyak Noll, & Chikindas, 2011). The acidic environment does not affect the commensal organisms that already live in these areas because they have evolved to live in these conditions. This hostile environment prevents bacteria from being able to colonize and keeps the healthy

commensals stable. Colonization resistance is main function of the microflora and vital to prevent the colonization of pathogenic bacteria in or on the body. Without this vast microflora, pathogens would be able to freely attach to cells and try to penetrate the epithelial membrane to cause infection.

Blood Brain Barrier

The microflora can also play a profound role in the maintenance and permeability of the brain barrier (Wang & Wang, 2016). Many of the studies done on this topic have compared the functions of the brain with germ free mice and regularly colonized mice. The studies done have shown that the microflora can have profound effects in cognitive development and what hormones are produced in the brain (Wang & Wang, 2016).

In one study different germ free mice were compared to normally raised mice to look at how they respond to stress (Cryan & Dinan, 2012). The study revealed that the germ free mice reacted drastically different when put under different stressful situations. In a study done mice were put under mild restraint and their stress responses were different. The results showed the germ free mice released higher amounts of adrenocorticotropic hormone and corticosterone hormone then did the normally colonized mice (Sudo et al., 2004). This means that the mice reacted more negatively to stress and released physically different hormones then normal mice. In younger mice if recolonized with normal gut microflora their brain behavior reverted back to that of normal mice. However as the mice grew older they did not recover to the levels of normal responses. This supports the idea that these microflora play a large role in the bran development process and there is a critical window early in development where it is most important (Cryan & Dinan, 2012).

Food Allergy

Food allergy and other disease are becoming much more prevalent. Since 1997 the incidence of peanut allergies has tripled from .4% to 1.4%. The incidence of other allergies has also increased and now affects 6% of children under 5 years old (Gigante et al., 2011). The causes of this drastic change are environmental and can be due to the development of the microflora. The scientific community is beginning to find evidence this shift is due to limited exposure of infants to certain bacteria and the overuse of antibiotics. As mentioned earlier tolerance is an important role of the microflora. As an infant develops the Peyer's Patches and other areas such as the GALT are developed (Rachid, 2016). With limited microflora these areas are underdeveloped and not able to develop tolerance as effectively. As a result these areas are not able to sample as many antigens and allergies can occur to non-harmful antigens such as peanuts.

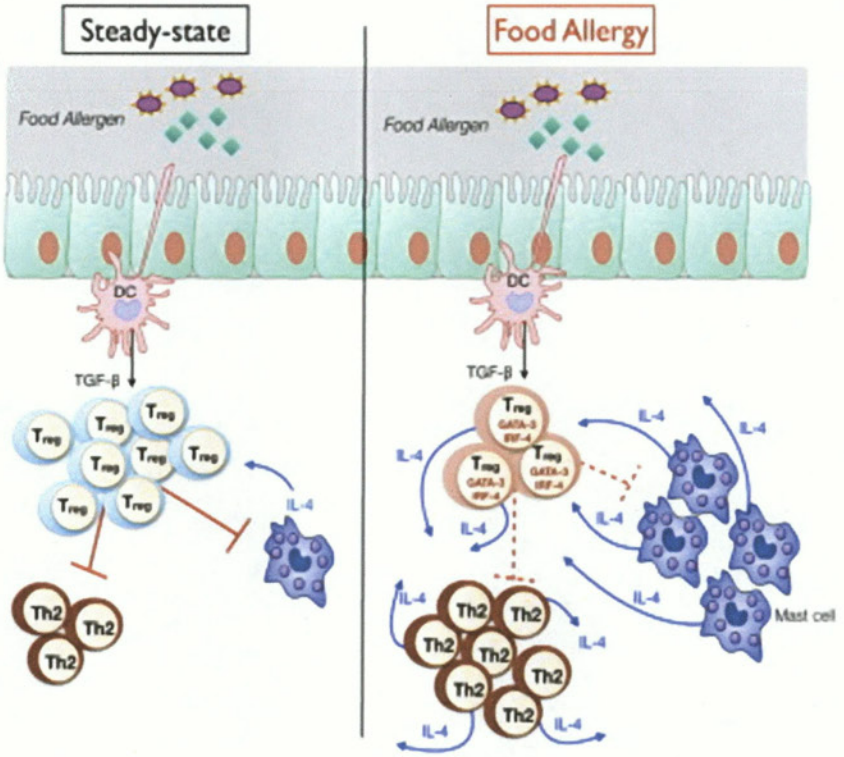

Figure 4: This image shows the difference in response between a person with a food allergy and a person without a food allergy (Noval Rivas et al., 2015).

One of the essential cells involved in tolerance is the T regulatory cells. These cells help to regulate the immune system and have anti-inflammatory properties. In mice that are raised in a germ free environment this is less IL-10 which is a cytokine that promotes the differentiation of T regulatory cells (Roncarolo, Bacchetta, Bordignon, Narula, & Levings, 2001). Through development these T regulatory cells make sure that the immune system does not overreact to different antigens such as food particles. In figure 4 the depiction with the food allergy shows the dendritic cells presenting the antigen to the T regulatory cells. In the individual without the food allergy mast cells are not recreated to the site. In the one with food allergy the T regulatory cells are not tolerant to the antigen and produce a systemic response causing harmful inflammation (Pellerin, 2014). The germ free mice also showed lower levels of IL-17, which is also involved in helping to make T regulatory cells. As these mice developed without these cytokines they are were not able to acquire oral tolerance that promoted the development of allergies. In this study the presence of *Clostridia constorium* in infant mice protected the mice from developing an allergy to foods (Rachid, 2016). The increasing prevalence of food allergy can be associated with a changing microflora. Microflora play a key role in tolerance to non-harmful food antigens during development and could maybe help prevent food allergies from developing.

Microflora and other Disorders

One study done on mice showed a link between the dominant microflora and obesity. By looking at the microflora composition predictions could be made if they were

predisposed to obesity. In the study it showed that a mouse high percentages of enterococci and lactobacillus and low *Bacteriodetes* would be predisposed to obesity (Mozeš, Bujňáková, Šefčíková, & Kmeť, 2008). Infants that are breast fed in humans tend to have a healthier weight because of a higher number of *Bacteriodetes* (Bergmann, 2003).

One other clinical use of the microflora is the treatment of *C. difficile* with a fecal transplant. In the study the patients had *C. difficile* and multiple rounds of antibiotics were not killing the bacteria. Instead a fecal transplant from a healthy individual with a healthy microflora was transplanted into the infected individual. This cured the patient of the disease as well as colonized the patient with a healthy gut microflora. This transplant also stopped the use of antibiotics that was causing the bacteria to start to develop a drug resistance (Fecal Microbiota, 2015). In a similar study patients with Irritable Bowl Syndrome (IBS) were compared with healthy individuals. With these patients the total number and diversity of anerobes were compared and the patients with IBS had less anerobes and diversity. (Madden et al., 2001). In another study probiotics were used to help alleviate the symptoms of IBS. In this study it showed that people who took probiotics with IBS had less abdominal pain as compared to no probiotics (McFarland & Dublin, 2008). By altering the microflora both IBS and *C. difficile* patients have shown improvements or been cured as a result.

Conclusion

The microbiome plays a crucial role in so many functions of the body. The immune system is dependent on a healthy microflora to function with proper efficiency (Blaut, 2006). These microbes are not meant to be feared and work to benefit the body as

well as themselves. These microbes should be viewed as having a symbiotic relationship with humans because both humans and microbes benefit from the relationship. Many different disorders can be prevented with a healthy microflora and hopefully new treatments are developed in the future using these microbes as allies. The overuse of antibiotics and limited exposure to different bacteria is limiting the diversity and health of our microflora. Each person has their own unique microflora that has developed and interacts with their body. Much more research still needs to be done on the organisms to see exactly how they interact and what other potential benefits they can have.

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